

# Hungry females show stronger mating preferences

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Female mating decisions that are based on condition-dependent traits, such as male nutritional state, may be associated with a female's own condition. In the swordtail fish, *Xiphophorus birchmanni*, females prefer the chemical cues of well-fed males to cues of food-deprived males. Here we show that this preference is significantly stronger in females when they were experimentally food deprived than when they were well fed. Our results suggest that if females have limited access to food resources, and are themselves food deprived, they will attend to cues indicating male nutritional condition more than when environmental conditions allow for greater access to food. Furthermore, not only is the slope of the preference function condition dependent but also, in all trials, the latency to respond to the presented stimuli was shorter in food-deprived females, suggesting that responsiveness to environmental cues is condition dependent as well. Under natural conditions, females of many species likely experience variation in resource availability. Thus, we predict that covariance between the strength of female preferences and resource availability may be widespread and may represent a common source of female preference variation within and between populations. *Key words*: mate choice, pheromone, Poeciliidae, sexual selection, trophic environment. [*Behav Ecol*]

Female mate choice is most often studied at a single point in time, but increasing evidence suggests that mating decisions may vary over the course of a female's lifetime. Mating preferences, for example, can vary as females age (Moore PJ and Moore AJ 2001; Coleman et al. 2004) or with changes in the local environment (Endler and Houde 1995; Fisher et al. 2006) or predation risk (Berglund 1993; Godin and Briggs 1996). Access to resources can also affect a female's preference for male traits. For example, female black field crickets (*Teleogryllus commodus*) reared on a high-protein diet are more sexually responsive and express stronger preference functions for 2 aspects of male calls (Hunt et al. 2005). In contrast, female guppies (*Poecilia reticulata*) reared on a high-carotenoid diet have weaker preferences for the carotenoid content of male orange spots (Grether et al. 2005). These studies suggest that a female's sensitivity to a trait indicating male quality can be regulated by her lifetime access to resources. In most natural environments, resource availability fluctuates over brief temporal scales (MacNally 1995). The slope of a female's preference function, or the rate at which the strength of response from the female varies in relation to the male trait (Jennions and Petrie 1997; Widemo and Saether 1999), may vary on a shorter temporal scale, based on current or recent environmental conditions.

In this study, we investigated how female mate choice varies as a function of food availability in a swordtail fish, *Xiphophorus birchmanni*. We experimentally manipulated female access to food and evaluated whether female nutritional state influences mate preference for male cues indicating nutritional condition. Female *X. birchmanni* use chemical cues both in interspecific (Fisher et al. 2006) and intraspecific mate choice, preferring the cues of well-fed males over those that have been food deprived (Fisher and Rosenthal forthcoming). Under natural conditions, *X. birchmanni* live in lowland, freshwater streams in the Río Panuco basin of eastern Mexico that are

subjected to seasonal flooding and anthropogenic disturbance (Fisher et al. 2006), which likely influence resource availability. Fluctuating environmental conditions may have influenced the evolution of the preference for well-fed males in *X. birchmanni* because a male's ability to obtain resources should be a better indicator of quality in a depauperate environment than in a resource-rich environment. We hypothesized that as food availability decreases, it should become more informative for females to use male nutritional condition as an indicator of quality.

## METHODS

All subjects were wild-caught adults from the Río Garces, Hidalgo, Mexico (20°57'22"N 98°16'48"W). In captivity, animals were housed in large (200l) aquaria, maintained on a 12:12 h light:dark cycle and fed twice a day on TetraMin flake food ad libitum supplemented with live brine shrimp.

We used 20 females and 10 males for this study. The females were divided into 2 groups of 10 each and arbitrarily assigned group A and group B, and each group was placed into their own well-planted 80-l aquarium. During the testing period, group A females were fed 0.2 g of TetraMin at 9:00 AM, 11:00 AM, 1:00 PM, and 3:00 PM (wild swordtails are most active and feed during this midday period [HS Fisher and GG Rosenthal, personal observation]) and were considered "well-fed" females, and group B females were given no food for 5 consecutive days and considered "food-deprived" females (Plath et al. 2005; Fisher and Rosenthal forthcoming). The males were also divided into 2 groups of 5 each and assigned group A and group B, and each group was placed into their own well-planted 40-l aquarium. Group A males were considered "well-fed" males and fed 0.1 g of TetraMin at the same intervals as the well-fed females (the amount of food was reduced to half to account for half as many animals), and group B males were given no food for 5 days and considered "food-deprived" males. After this period of 5 days, female preference tests were conducted (see below) on 20 of the females, and all animals were returned to a normal feeding schedule for 10 days. We then repeated the feeding regime described above except group B females and males were well-fed, and group A females and males were food-deprived, and again conducted female preference tests after

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Received 30 January 2006; revised 3 July 2006; accepted 12 July 2006.

five days. In this design, all females were tested as both well fed and food deprived in order to serve as their own control for pairwise analysis. During the study, all animals were closely monitored, and no disease or deaths occurred.

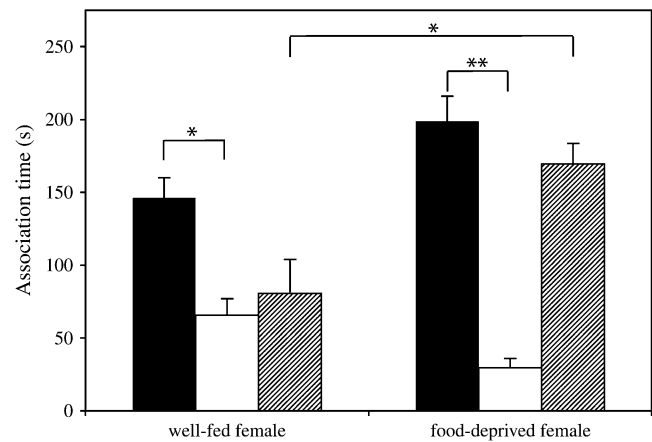
In *X. birchmanni*, visual cues are important in mate attraction; however, chemical cues are both necessary and sufficient for conspecific mate recognition in other *Xiphophorus* species (Crapon de Caprona and Ryan 1990; Hankison and Morris 2003), and female *X. birchmanni* attend to variation in chemical cues among males (Fisher and Rosenthal forthcoming). To remove potentially obscuring effects of variation in male visual cues, we isolated chemical cues for presentation to females (McLennan and Ryan 1997, Fisher et al. 2006). Chemical stimulus preparation and preference tests closely followed methods in Wong et al. (2005) and Fisher and Rosenthal (Forthcoming). Stimulus water for female mate choice experiments were prepared by moving 5 males into a single 20-l collection aquarium, which was adjacent to a 20-l aquarium containing 5 conspecific females to provide them visual stimulation. Males remained in the collection aquarium for 3 h. Preference tests were conducted in an aquarium (length  $\times$  width  $\times$  height = 75  $\times$  30  $\times$  30 cm) divided lengthwise into 3 equal sections by lines drawn on the sides of the tank and filled with 40 l of water. Each test tank had 2 stimulus delivery systems located at either end of the tank carrying stimulus water from well-fed males and food-deprived males. Each stimulus delivery system consisted of 2.5-mm silicone tubing fixed to the bottom of a collection aquarium with a suction cup on one end and 1 cm above the waterline of the test tank on the other end. The collection aquarium was raised above the test tank to aid the flow of stimulus water into the test tank, and a valve controlled the flow to a rate of 5 ml/min.

Each female was acclimatized for 30 min in the test tank containing testing water; stimulus flow was then initiated. A preference test began once the female had passed into both side compartments and continued for 300 s. If the female did not visit both compartments within 300 s, the trial was ended. Variables scored were 1) latency, the time from initiation of stimulus flow until female comes within 25 cm of each stimulus outflow and 2) association time, the total time spent within 25 cm of stimulus outflow. Association time is widely used in studies of mate choice in fishes and is the standard measure of mating preferences in poeciliids, including swordtails (see Wong et al. 2005 and references therein). In a closely related species, *Xiphophorus nigrensis*, association time in laboratory trials is a strong predictor of association in open field trials and of observed mate choices in the wild (Ryan et al. 1992). Association time is thus a meaningful estimate of mating preference. All statistical tests performed were 2 tailed.

In order to determine if females prefer food odor cues over cues produced from well-fed males, we tested 10 females for their preference for water containing food cues and well-fed male chemical cue. We ground 1 g of TetraMin food with a ceramic mortar and pestle until it was the consistency of a powder. The food was then added to 1 l of filtered water and shaken vigorously for 1 min. The solution of food was treated similarly to the male chemical cues and used in female preference tests. Both well-fed females and food-deprived females from the above experiment were tested following the preference tests procedure used in the mate choice tests.

## RESULTS

We found that both well-fed and food-deprived focal females preferentially associated with the chemical cues from well-fed males over cues from food-deprived males (paired *t*-test: well-fed females,  $t = 2.71$ ,  $df = 19$ ,  $P = 0.013$ ; food-deprived females,  $t = 4.91$ ,  $df = 19$ ,  $P < 0.001$ ; Figure 1). There was, however, no



**Figure 1**

Mean association time + standard evaluation (SE) of well-fed and food-deprived focal females to the chemical cues of well-fed (black bars) and food-deprived (white bars) male chemical cues. Gray bars indicate mean net association time (time spent with well-fed male chemical cues – time spent with food-deprived male chemical cues) + SE of females. \* $P < 0.05$  \*\* $P < 0.001$ .

significant difference between female latency to either of the male cues in both well-fed female and food-deprived female tests (paired *t*-test: well-fed females,  $t = 1.83$ ,  $df = 19$ ,  $P = 0.082$ ; food-deprived females,  $t = 0.350$ ,  $df = 19$ ,  $P = 0.729$ ).

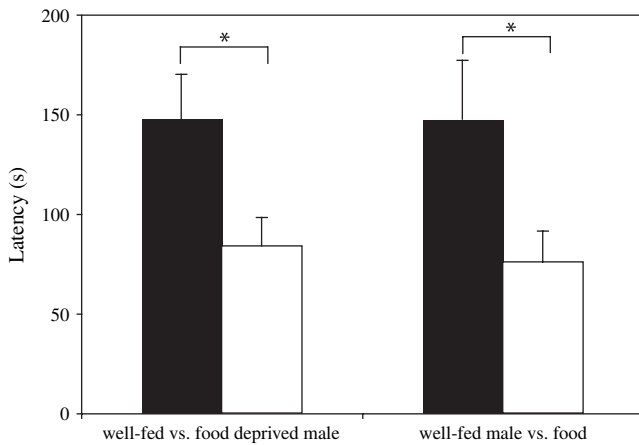
In support of our hypothesis that food-deprived females should show a stronger preference than well-fed focal females for well-fed males, we found that the net preference for well-fed males (association time with well-fed male stimulus – association time with food-deprived male stimulus) of food-deprived focal females was significantly greater than well-fed females (paired *t*-test:  $t = 2.59$ ,  $df = 19$ ,  $P = 0.029$ , Figure 1).

When females were tested for their preference for chemical cues from food over those of well-fed males, we found that both well-fed females and food-deprived females showed a strong preference for food (paired *t*-test: well-fed females,  $t = 2.55$ ,  $df = 9$ ,  $P = 0.030$ ; food-deprived females,  $t = 3.72$ ,  $df = 9$ ,  $P < 0.001$ ). In these trials, however, the strength of their preference (the net preference) did not differ between well-fed females and food-deprived females (paired *t*-test:  $t = 0.398$ ,  $df = 9$ ,  $P = 0.699$ ).

We found that the total latency (time it took females to respond to both stimuli) of well-fed females was significantly longer than the total latency of food-deprived females (paired *t*-test:  $t = 2.35$ ,  $df = 19$ ,  $P = 0.029$ , Figure 2). Likewise, when focal females were presented with food stimuli and well-fed male cues, we again found that well-fed females responded more slowly to both cues (paired *t*-test:  $t = 2.38$ ,  $df = 9$ ,  $P = 0.041$ , Figure 2).

## DISCUSSION

Female condition had a pronounced effect on female mate choice, in line with our prediction that food-deprived females should show stronger preferences for chemical cues associated with male nutritional state. We suggest that these results reflect a behavioral response to fluctuating environmental conditions. If a swordtail female is unable to obtain food over a 5-day period, it likely reflects an environment where resources are scarce. In a food-limited environment, males that are able to find food, and/or those that have ample fat reserves, are better suited for coping with a fluctuating environment (Plath et al. 2005). In *X. birchmanni*, the chemical cues produced by males are dependent on nutritional state and are used by females in mate choice (Fisher and Rosenthal forthcoming). The results



**Figure 2**  
Mean total latency + SE of focal females in preference tests where the presented stimuli were well-fed versus food-deprived male cues, or well-fed male versus food cues. In both cases, black bars indicate total latency of well-fed focal females and white bars indicate total latency of food-deprived females. \* $P < 0.05$ .

from the present study suggest that the strength of this preference changes as a function of females' access to food.

Our results further suggest that this change in female preference function reflects a plastic behavioral response to environmental conditions. All females were tested as well-fed and as food-deprived females, and our results therefore reflect a preference function that varies by feeding regime. Furthermore, the 2 treatments (well fed and food deprived) were conducted within days of one another, suggesting that female preference is dynamic over relatively short time periods.

The difference in preference between well-fed and food-deprived females may, alternatively, reflect a more general tendency for food-deprived females to respond to cues in the environment. We found that the total latency of well-fed females was significantly greater than that of food-deprived females in all sets of trials (Figure 2). Our data suggest that the food-deprived females are more motivated to explore the environment, likely in search of food. Another possible explanation is that food-deprived females may attend to the cues of well-fed males faster because they are attracted to food excretions emitted by the males. This explanation is unlikely in *X. birchmanni* because females show no preference for chemical cues of well-fed females over cues of food-deprived females; the preference is specific to male cues (Fisher and Rosenthal forthcoming). Further, we did not observe a difference in preference between well-fed females and food-deprived females when given the choice between food cues and well-fed male cues, which indicates that female nutritional state does not affect their preference for these cues, only the time that they take to respond to cues.

In this study, female latency was dependent only on female condition rather than on the type of cue being presented. Females responded equally fast to food cues and male cues, but food-deprived females responded significantly faster than well-fed females to both types of stimulus. Our results are in contrast to a recent finding on food deprivation in meadow voles (*Microtus pennsylvanicus*), where an interruption in food availability of only 6 h had inhibitory effects on female initiation and maintenance of social interaction with males (Pierce et al. 2005). The difference in response between swordtail fish and meadow voles likely represents differences in short-term energy requirements between the species; a longer period of food deprivation in swordtails might produce a similar response.

In this study, we present data suggesting that females use their current nutritional state as an indicator of resource availability and that the slope of females' preference functions for well-fed males reflects current environmental conditions. Female mate choice is thus sensitive to environmental variation on a relatively short timescale. The strength of sexual selection may decrease if individuals vary in their mate preferences or if signals are assessed under different environmental conditions (Candolin 2003). Thus, mating preferences that change as a function of resource availability may critically affect a population's evolutionary trajectory.

We thank Francisco García de León, Bob Wong, Rhonda Struminger, and Juventino Tovar Ortiz for their assistance in the field as well as Seth Coleman for his comments and discussion. We are indebted to the Mexican federal government for permission to collect fish. This research was supported by National Science Foundation grant IOB-0447665 to G.G.R. H.S.F. was supported by a Palmer-McLeod Fellowship.

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